THE EFFECTS OF ILLUMINATING GAS ON ROOT SYSTEMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 205

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(WITH NINE FIGURES)

Introduction

The injurious effects of illuminating gas upon trees and shrubs have been testified to by numerous observers. In 1864 GIRARDIN (5) reported severe injuries to trees in several cities of Germany which he attributed to escaping illuminating gas. Similar observations have since been recorded by many writers, among whom are VIRCHOW (17), KNY (8), SPÄTH and MEYER (14), EULENBERG (4), WEHMER (18), SHONNARD (13), and others.

The shade tree commissions of every city find themselves face to face with this serious problem. The trees of our city streets and parks are unusually subject to the various tree-injuring agencies, the chief of which are insects, fungi, and atmospheric and soil impurities. The two latter factors are particularly characteristic of the troubles of city trees. The problem of gas injuries, therefore, is one of considerable economic importance. City foresters should know the exact cause of any tree death, not only to enable them to provide means for future protection, but also in order to determine with whom responsibility rests for the present financial losses. They should therefore be able to say with certainty whether or not a tree has been killed by gas in the soil. At present this is no small undertaking, because there are few, if any, reliable symptoms known by which one may differentiate with certainty gas injuries from those due to several other causes. For example, fungi sometimes quickly become prominent in a tree injured by gas, as purely a secondary effect (see KNY 8 and STONE 15); but in a case like this the primary injury might easily be attributed to the fungi. It is claimed that characteristic odors often accompany gas poisonings; sometimes in the roots as well as in the soil; in fact, these odors form the chief diagnostic characters. STONE (15) claims much for them, and it is probably true that in many cases they furnish some evidence. However, many instances are known where illuminating gas odors have not given sufficient evidence for fixing responsibility. A rational system of diagnosis of injuries in city trees would be of value and would be welcomed by all parties concerned. No such system, from the gas injury standpoint, is possible, owing to the lack of definite knowledge concerning the effects of the constituents of illuminating gas under various controlled conditions.

The experimental work previously done on gas injuries to root systems will be briefly summarized. KNY (8) passed known amounts of illuminating gas through the soil at the roots of a maple and two linden trees. Among the symptoms of injuries recorded were the bleaching and final fall of leaves and the appearance of a blue coloration in the xylem of the roots. Finally the trees died. SPÄTH and MAYER (14) passed small amounts of gas into soil in which grew a number of species of trees and shrubs. General death resulted, but otherwise the only symptom recorded was the yellowing of the leaves. BÖHM (1) grew willow slips in water into which he had passed a stream of illuminating gas. The short roots produced soon died, but the slips themselves lived for three months. Potted plants of *Fuchsia* and *Salvia* died after gas had flowed to their roots for four months. Again, he found that when gas had been passed through a soil for a long period of time, this soil became very toxic to seedlings germinated in it. Also a *Dracaena* transplanted to this soil became dry and died in ten days. MOLISCH (10) found that roots of corn increased in diameter and were bent in certain concentrations of illuminating gas. SHONNARD (13) noted exudation of sap from the trunk and branches of a lemon tree treated with gas in the soil. RICHARDS and MACDOUGAL (11) found that carbon monoxide and illuminating gas retarded the rate of elongation of roots of *Vicia Faba*, sunflower, wheat, rice, etc. Swelling also appeared in the leaf sheaths of wheat, being somewhat more pronounced in illuminating gas than in carbon monoxide. Such increases in thickness were largely due to the enlargement of the cortical cells. In some cases, however, the cambium seemed to have become more active. Recently STONE (16) has reported proliferations of tissue at the lenticals of willow slips growing in water which had been charged with illuminating gas. He also noted a rapid proliferation at the cambium in stems of *Populus deltoides* due to the influence of gas.

Another important phase of the gas injury problem is that after trees have been killed by gas, a question arises regarding the safety of resetting trees where the dead ones have been removed, assuming, of course, that the gas leak has been located and stopped. It seems to be the general opinion that resetting should only be done either after a considerable time has elapsed, or after large amounts of the old soil have been removed and replaced by fresh soil. Neither of these methods of procedure is entirely satisfactory; the first involves great loss of time, the second is expensive. The practicability of resetting trees in any given case is often determined only by the crude method of smelling a handful of soil taken from the place of injury, and if the odor of gas is still present, resetting is deemed unsafe. One is thus led to ask whether the odor itself is a true index of the toxicity of the soil to the roots of plants.

The investigation reported below was undertaken with the two problems in mind: (1) that of determining some of the effects of illuminating gas on root systems, having in mind the securing of further diagnostic characters of gas poisoning; and (2) whether the chief causes of injury are those constituents of illuminating gas which are readily absorbed by the water film of the soil particles, or those which remain mainly in the soil interstices (not being so readily soluble).

Methods and materials

The illuminating gas used was the so-called "water gas" from the Chicago Gas Light and Coke Company's system. Along with the illuminating gas experiments, many parallel ones were carried out with an ethylene-air mixture. The Chicago illuminating gas contains 2–6 per cent ethylene; therefore, to facilitate comparison between the ethylene alone and the ethylene of the illuminating gas, the ethylene of the mixture above was made to constitute 4 per cent (by volume). Thus, volume for volume, the ethylene-air mixture and the illuminating gas contained approximately the same amount of ethylene. In the following experiments, where the term "ethylene in corresponding concentrations to the illuminating gas" is used, it refers to the ethylene content of the latter gas. For example, the ethylene concentration corresponding to an illuminating gas concentration of one part gas to four parts of air ("1:4") would be one part ethylene to 100 of air ("1:100"). Such parallel experiments were considered important owing to the fact that ethylene has been found to be by far the most toxic constituent of illuminating gas for the aerial organs of several plants (see CROCKER and KNIGHT **2**, KNIGHT and CROCKER **7**, and HARVEY **6**).

The experiments were primarily arranged so as to yield evidence with regard to the two phases of the second problem above. The methods will be described separately and in the order named. Investigation of the second problem should yield data with regard to the first.

A. THE TOXICITY OF THE CONSTITUENT OF ILLUMINATING GAS ABSORBED BY THE SOIL

Good potting soil in 10–20-liter cans was treated with illuminating gas by allowing the gas to flow through at a definite rate, at room temperature, for varying lengths of time. The rate of flow was approximately 2 liters per hour. The time periods were from 30 hours to 20 days, hence the lots of soil received 40–1000 liters of gas. The moisture content of the soil was kept as near the "optimum" as possible. In one experiment 10 liters of soil received gas at the rate of about 0.2 liter per hour for 68 days. In another experiment 8 liters of soil received gas at the rate of 2 liters per hour for 53 days, and was kept at a temperature of 1–5° C. throughout the period. The purpose of this soil treatment was to allow the soil particles to absorb as much of the gas constituents as possible. In the case of treatment at low temperature, it was the intention to allow still better opportunity for condensation of substances on the soil particles. After stopping the flow of gas, the soils were removed from the cans and thoroughly stirred in pure air to free them from the gas in the interstices. They were then taken to the greenhouse, placed in shallow boxes, and planted to 41 different species of plants, representing 18 families. Controls were maintained throughout. The seeds were allowed to germinate and grow for periods of 25–60 days.

B. THE EFFECT OF ILLUMINATING GAS ON ROOTS WITH NO SOIL PRESENT

1. *With roots alone exposed to gas*

In order to expose the roots without exposing the shoots, the following method was employed. Moist-air chambers were made from 8-liter, wide-mouthed bottles; 6–8 short glass tubes were inserted in the corks of each of these bottles so that the tap roots of young seedlings of *Vicia Faba* could be admitted to the chamber. The space between a root and the sides of a tube was sealed by means of a short length of pure gum tubing which had been previously fitted to the outer end of the glass tube. Definite amounts of illuminating gas or ethylene were admitted through a small glass tube reaching to the bottom of the bottle. The pressure resulting from the addition of the small volume of gas was relieved through a second short tube.

2. *With entire plant exposed to gas*

Seeds of tomato, radish, and mustard were allowed to germinate, and when the hypocotyls had reached a length of 0.5 cm. they were transferred to flower pots under bell jars provided with water seals. The seedlings were fixed to the rims outside of the flower pots, so that the roots in growing would hang free in the air. Definite amounts of illuminating gas could be easily added.

C. EFFECT OF ILLUMINATING GAS ON ROOTS GROWING IN SOIL MEDIUM

1. *Quantitative tests*

In these tests young seedlings (two or three months old) of *Catalpa speciosa*, *Ailanthus glandulosa*, and *Gleditschia triacanthos* were used. A few days before the beginning of the experiments, the seedlings were transplanted from the pots, in which they had germinated, to large battery jars filled with coarse quartz sand. Two glass tubes for admitting gas were thrust into the sand and then the roots and glass tubes were sealed in with a vaseline-paraffin mixture by the BRIGGS and SHANTZ method. The total volume of the interstices (allowing for a definite water content) was previously determined, and upon the basis of this volume, known concentrations of gas could be secured. The duration of experiments was 5–21 days. Gases were changed every three days by drawing pure air through the jars for 10–15 minutes by means of an aspirator, and new gas added. Parallel experiments with ethylene were also run in many cases.

2. *Qualitative tests*

In this series of experiments it was desired to subject a number of plants to illuminating gas under conditions met with in the field where leaking gas mains are involved. Potted woody plants, including 36 individuals of 11 species, were used. Illuminating gas was allowed to pass slowly into the soil around the roots. Meanwhile symptoms of injury were noted. When the plant had died, or become seriously injured, or after a certain time had elapsed, the roots were washed clear of soil, and careful examinations made. The rate of gas flow was often less than one-eighth liter per hour.

Results and discussion

The results will be presented in the order in which the methods were described.

A. EFFECT OF ILLUMINATING GAS ON SEEDS PLANTED IN SOIL PREVIOUSLY TREATED WITH GAS AND THEN AERATED

In all cases, at the time of the planting of the seeds, the treated soil gave an exceedingly strong odor of illuminating gas. Of the 41 species planted, 5 failed to germinate, but the failure was in both the controlled and the treated soils. Throughout the 25–60 day period, the aerial portions of the seedlings were watched for signs of injury. However, it was found that the plants in all the treated soils gave no superficial evidence of injury. All seemed perfectly normal. At the end of the period the plants were taken up, their roots washed free of soil, and examinations made. Only two species gave any evidence of injury; these were cotton and lupine. In them the root systems were perhaps somewhat less developed in the treated soils, and in the cotton there appeared to be a greater development of anthocyanin in the treated soils. At the close of the experiments, the treated soils always gave a noticeable gas odor and in most cases the odor was very strong.

The results of these experiments would indicate that of the constituents absorbed from illuminating gas by soil, those which give the odor to the gas are very prominent, and when once absorbed are held for extended periods, even after the soil is freely exposed to the air. But the most important fact from the standpoint of the question in hand seems to be that these odorous constituents are evidently not extremely toxic to plant roots growing in the same soil. The plants tried in such soils included a wide range in regard to relationship, and also they were taken at what is considered the critical stage, that is to say, the germinating and young seedling stages. Just what these odorous compounds are is an interesting question. The odor of any illuminating gas is probably the combined odor of a number of substances, for example, pyridine, thiophene, picoline, quinoline, cumene, cymene, and others. Very little is known concerning the effect of these on vegetation. CROCKER, KNIGHT, and ROSE (3) found cumene, thiophene, and pyridine were many hundred times less effective in reducing growth in the etiolated sweet pea seedlings than was ethylene.

The results of the experiments described indicate that the presence of a gas odor in soil is not an index of its toxicity for vegetation, and that odors would be valuable merely as a means of determining whether or not illuminating gas had been in the soil. With regard to using odors in diagnosis, CROCKER has suggested to us the possibility of distilling (at high temperature *in vacuo*) soil from places where gas injuries have been suspected, but where the odor even at the time is not discernible, thereby drawing off some of the odors previously held too firmly by the soil particles.

B. THE EFFECT ON ROOTS WITH NO SOIL MEDIUM

1. *With roots alone exposed*

Material, seedlings of *Vicia Faba*. Exposure period five days.

With illuminating gas.—(1) Concentration 1:4000; no effects were noted, evidently grew as well as controls; (2) concentration 1:400; growth in length somewhat retarded and two other strongly marked effects entered: (*a*) swelling back of the root tip, and (*b*) turning and coiling of root (fig. 1); (3) concentration 1:40; growth in length greatly retarded, also considerable swelling; coiling present, but the coils smaller and tighter.

With ethylene.—(1) Concentration 1:100,000 (that is, corresponding to ethylene of illuminating gas with (1) above); apparently no effect; normal growth ensued; (2) concentration 1:10,000; responses similar to those shown in fig. 1 but somewhat more pronounced; (3) concentration 1:1000; little or no growth in length; strongly swollen, no coils.

The parallel experiments with ethylene gave some evidence that the toxic effect recorded for the illuminating gas may be due to ethylene present in that gas because corresponding concentrations of illuminating gas and the ethylene-air mixture gave quite parallel results.

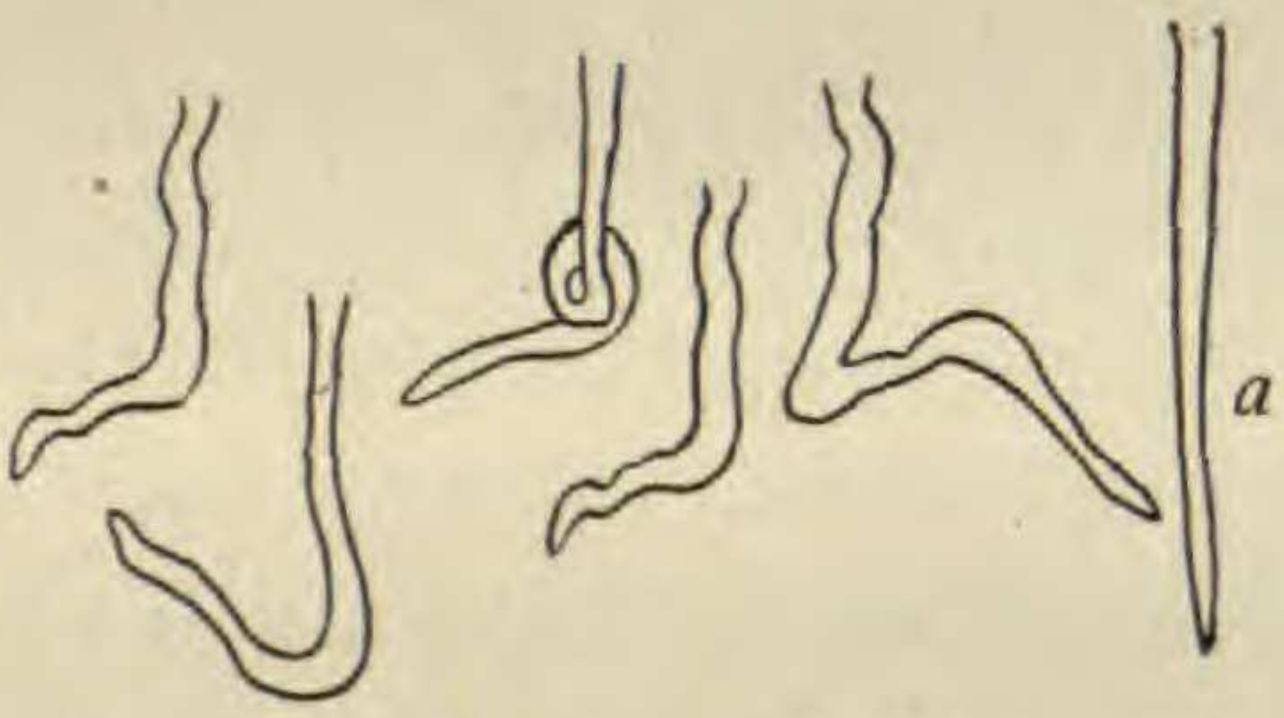


FIG. 1.—Outline of *Vicia Faba* roots, showing the effects of illuminating gas on growth; *A*, control.

2. *With entire plants*

Material, radish, mustard, and tomato. The roots and hypocotyls gave responses as follows:

Radish.—Exposure period 48 hours.

With illuminating gas.—(1) Concentration 1:500; bending of root evident (similar to those of fig. 1); no coiling; no swelling of hypocotyl or root; (2) concentration 1:5000; coiling and bending of root evident; no enlargement of hypocotyl or root.

Mustard.—Exposure period 48 hours.

With illuminating gas.—(1) Concentration 1:100; coiling and bending of root evident (similar to those of fig. 1); swelling of hypocotyl evident; no swelling of root; (2) concentration 1:11,000; bending of root slight; no other effect; (3) concentration 1:20,000; bending of root slight; no other effect.

Tomato.—Exposure period 3 days.

With illuminating gas.—(1) Concentration 1:500; swelling between stem and root (fig. 2); growth considerably retarded; (2) concentration 1:10,000; swelling as above, but less marked;

swollen zone longer and not so thick; more growth of hypocotyl; (3) concentration 1:100,000; little if any effect.

With ethylene.—(1) Concentration 1:12,500; short swollen knob between stem and root; growth greatly retarded; (2) concentration 1:250,000; swollen knob longer and not so thick as in (1); (3) concentration 1:2,500,000; little if any effect.

The response shown by the tomato seedling differs very markedly from that shown by the radish and mustard seedlings. While the roots of the radish and mustard seedlings show a coiling and

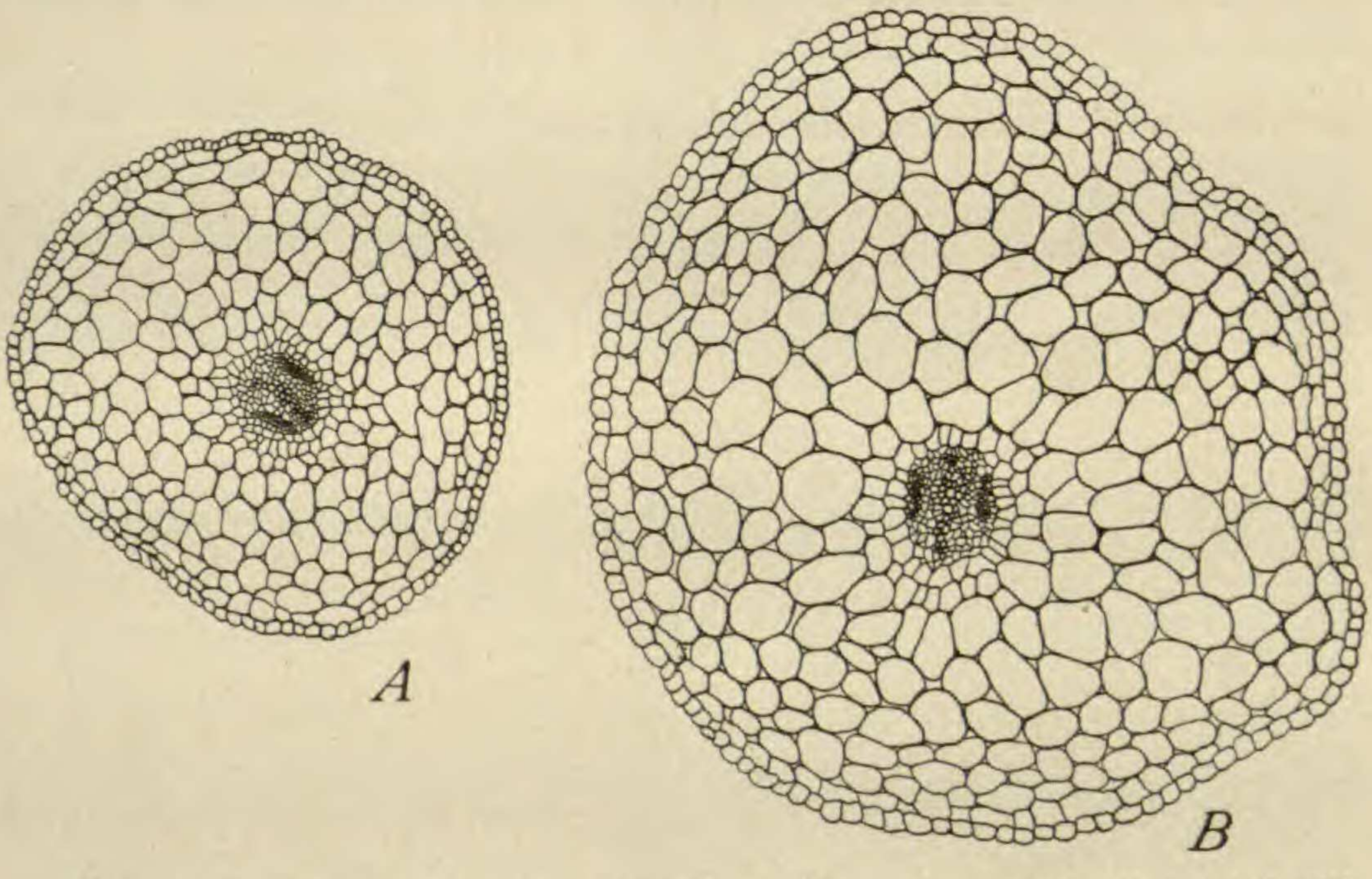


FIG. 2.—Sections of the root of tomato seedlings: *A*, control; *B*, treated with illuminating gas; ×18.

bending similar to those of the *Vicia Faba*, in addition to a slight swelling of the hypocotyl, the only response shown by the tomato seedling is a decided swelling of the hypocotyl and root at the point where the two join. With the tomato seedling the parallel experiments with ethylene again give some evidence that the toxic effect recorded for illuminating gas is due to the ethylene constituent of that gas.

The results recorded above for *Vicia Faba*, radish, and mustard show that injuries to roots may readily be brought about by placing them in an atmosphere containing small amounts of illuminating gas or ethylene. If we consider here also the results of the first series of experiments, it would seem that the chief causes of injury to root systems are those constituents of illuminating gas which are present mainly in the interstices of the soil, rather than those dissolved in the soil water.

The greatest danger in replanting where other plants have been killed by gas, seems to lie in the constituents remaining between the soil particles. If, therefore, a method was devised for quickly aerating this soil, trees might be safely planted at once without removing large amounts of soil.[1]

C. THE EFFECTS OF ILLUMINATING GAS ON ROOTS IN SOIL MEDIUM

1. *Quantitative tests*

a) *Catalpa speciosa* seedlings; illuminating gas; exposure period 8 days; concentrations 1:2000, 1:400, 1:200, 1:40, 1:20, and controls.

Stems and leaves showed no modifications; neither were there any strongly marked effects on the root systems. In concentrations 1:40 several roots gave indications of swelling 1–2 cm. back of the tips, while in concentrations 1:20 these swellings were very evident.

b) Same as above, with the exception of ethylene in place of illuminating gas but in corresponding concentrations (that is, 1:50,000, 1:5000, 1:1000, and 1:500).

No effect on stems or leaves. The responses with concentrations 1:1000 and 1:500 were like those above, but in addition, the tendency of the roots to coil at the tips (as noted in *Vicia Faba*, fig. 1).

c) *Catalpa* seedlings; illuminating gas; duration of exposure 21 days; concentration 1:4 and controls.

Stem and leaves gave no response. Roots of 1:4 gave swelling of main root extending from near the surface of the sand 4–7 cm. downward (fig. 3). This increase in amount was 2–3 times that of the normal thickness. The epidermis was often cracked and sloughed off in places. The drawing of fig. 4 shows that the swelling

[1] Perhaps this could be accomplished by passing pure air through the soil by means of a pipe thrust below the surface.

is brought about through an increase in size of the cortical cells, and particularly by rapid proliferation at the phellogen layer.

d) *Ailanthus* seedlings; illuminating gas; exposure period 15 days; concentrations 1:400, 1:10, and controls.

1:400 gave slight swelling in tap root near the surface of the soil; while in the 1:10 the leaves began falling after 5 days, and had all fallen before the end of the exposure. The tap roots were much swollen for 3–4 cm. below the surface of the soil.

e) Same as (*d*) but with ethylene instead of illuminating gas; concentrations 1:10,000 and 1:250.

Swollen zone of tap root was somewhat more pronounced in the 1:10,000 than in the 1:400 illuminating gas above. In the 1:250 all plants had lost their leaves within 8 days, and the tap roots were much swollen. The morphological nature of these swellings may be seen in figs. 5 and 6. In fig. 6 are two outlines of transverse sections of the tap roots of *Ailanthus* seedlings in the region where swellings take place; *A*, the control plant; *B*, a treated plant. Fig. 5 shows the detailed structures of the regions outlined in *A*

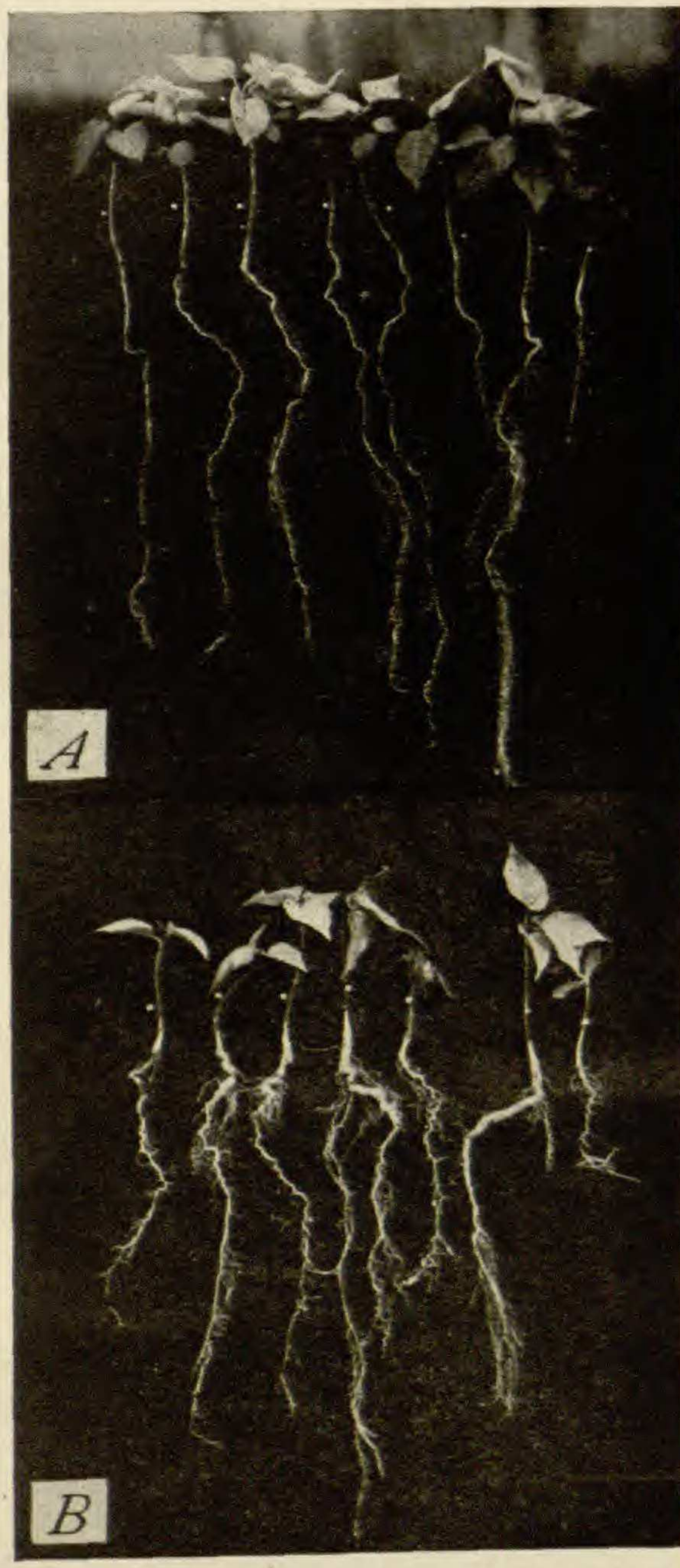
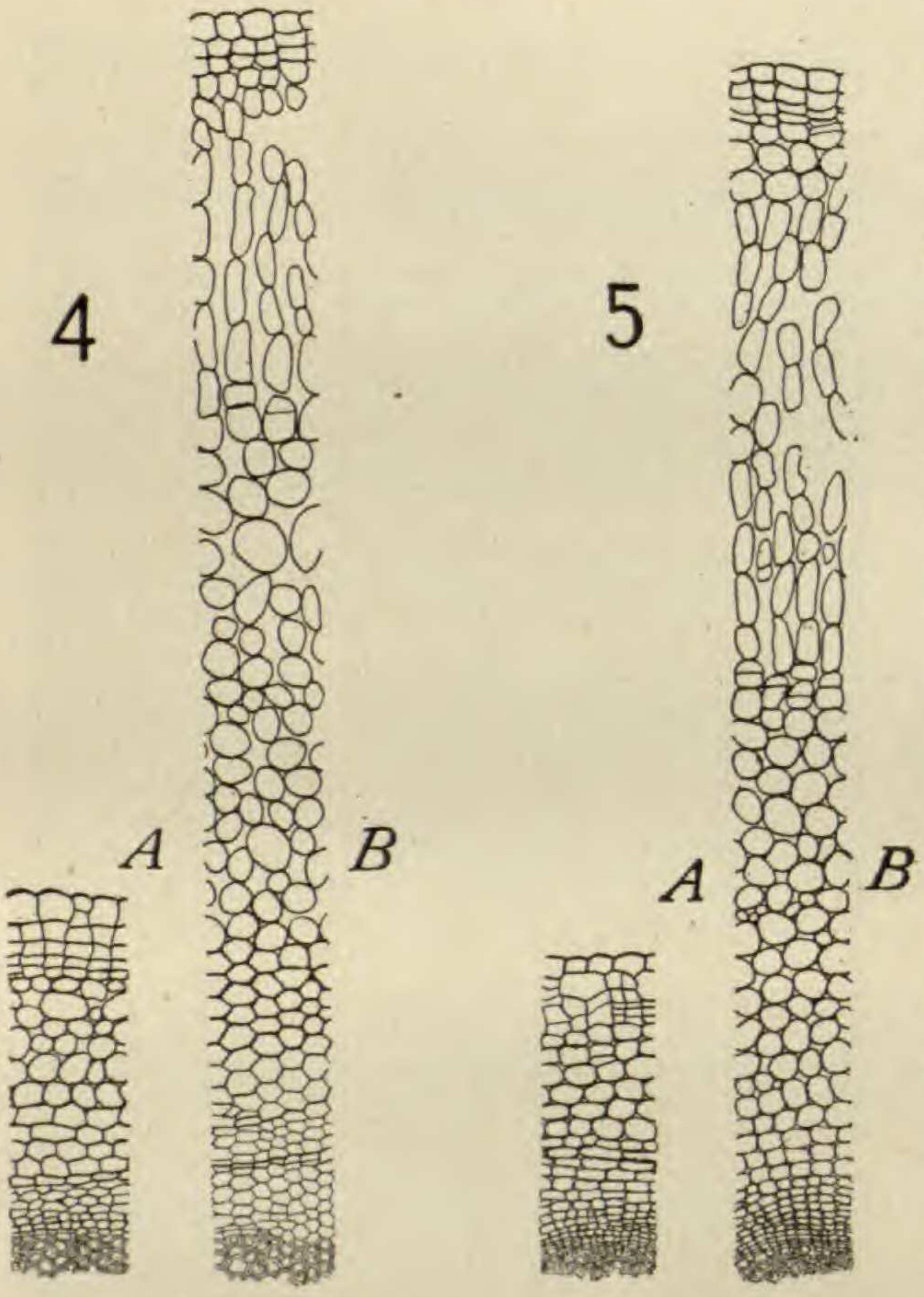


FIG. 3.—*Catalpa* seedlings: *A*, controls; *B*, treated with illuminating gas.

and *B* respectively of fig. 6. Through an examination of figs. 5 and 6 it becomes evident that the stelar region has remained unchanged, while the cortex, extending to the phellogen layer, has increased in thickness, partly through increase in the diameter of the cells, and partly through cell division. At the phellogen layer of the treated root, cell division has been rapid, resulting in the production of a loose tissue not present at all in the normal. The tissue lying outside the phellogen layer in the beginning has been only slightly modified in the abnormal root.



FIGS. 4, 5.—Fig. 4., section through the cortex of tap root of *Catalpa: A*, control; *B*, treated with illuminating gas; fig. 5, same for *Ailanthus* roots, showing detail of the tissue regions outlined in fig. 6; ×48.

f) A number of tests were carried out with *Gleditschia* seedlings, used in illuminating gas in various concentrations up to 1:3 (concentrations higher than 1:3–1:4 were not used for fear of oxygen becoming a limiting factor). Concentration 1:3 gave leaf fall, but no definite injuries were detected in the root system.

2. *Qualitative tests with potted woody plants*

When illuminating gas was passed rapidly through the soil of a potted plant, injuries might be observed the first day and death in all cases in the course of a few days. This is not at all surprising, because one should expect injury and final death to result as an effect of the shutting off of the oxygen supply, even though an inert gas (that is, nitrogen or hydrogen) be passed through the soil in this manner. Accordingly, illuminating gas could do great damage to vegetation independent of any direct toxic property. This view is advanced by KOSAROFF (9), who found that wilting took place if a stream of carbon dioxide or of hydrogen was passed through soil in which roots were growing. In all experiments where illuminating gas was passed into the soil rapidly enough to cause the death of the plant, the symptoms manifest in the aerial parts were of a type which would indicate that the injury might be due simply to the cutting off of the water supply as a result of injury to the root system, and not, necessarily, due to a conduction of toxic substances to them. With high evaporating conditions, the symptoms of gas injury (that is, wilting, yellowing, and falling of leaves in the aerial portions) always became manifest very much sooner than under conditions favoring a low water loss. It was found that when roots were killed quickly by using high concentrations of gas, few if any symptoms other than odors appeared which might enable one to diagnose the death as specifically gas poisoning. With regard to the injury to parts above soil, STONE (16) believes that "poisonous principles" are absorbed by the roots and conducted upward to the leaves, hence the yellowing and wilting would result as a direct poisoning of them.

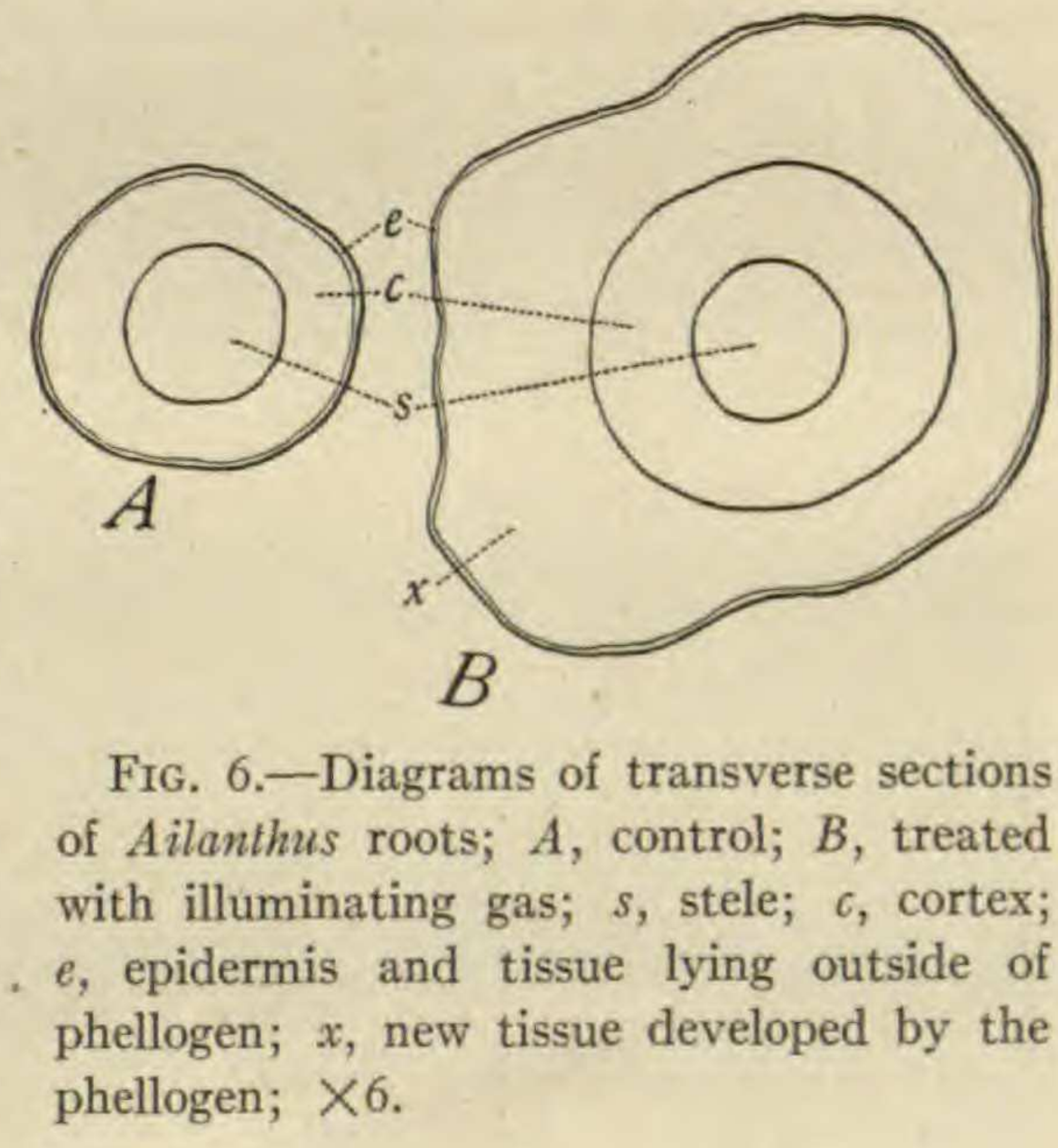


FIG. 6.—Diagrams of transverse sections of *Ailanthus* roots; *A*, control; *B*, treated with illuminating gas; *s*, stele; *c*, cortex; *e*, epidermis and tissue lying outside of phellogen; *x*, new tissue developed by the phellogen; ×6.

When illuminating gas was so regulated that it passed very slowly to the roots (sometimes as little as 40 cc. per day), a stimulation of the roots often took place, resulting in the development of new tissue resembling those reported from the quantitative experiments made with young tree seedlings. Stems were likewise affected when covered by soil or when slightly above the surface.

In stems this proliferation of cells became first evident at the lenticels by their increase in size and the protruding of white tissue, as shown in the lower portion of the stem in fig. 7. RICHTER (12) has noted a similar proliferation of tissue at the lenticels of *Vicia villosa* under the influence of tobacco smoke. STONE'S similar results have been cited. In the roots this tissue was abundantly developed in some cases, particularly in *Hibiscus*, which is shown in fig. 7, where *B* and *C* are plants which were subjected to a slow stream of illuminating gas for a period of 30 days. Fig. 8 shows detailed structure of these proliferations in the roots of *Hibiscus*. The cork

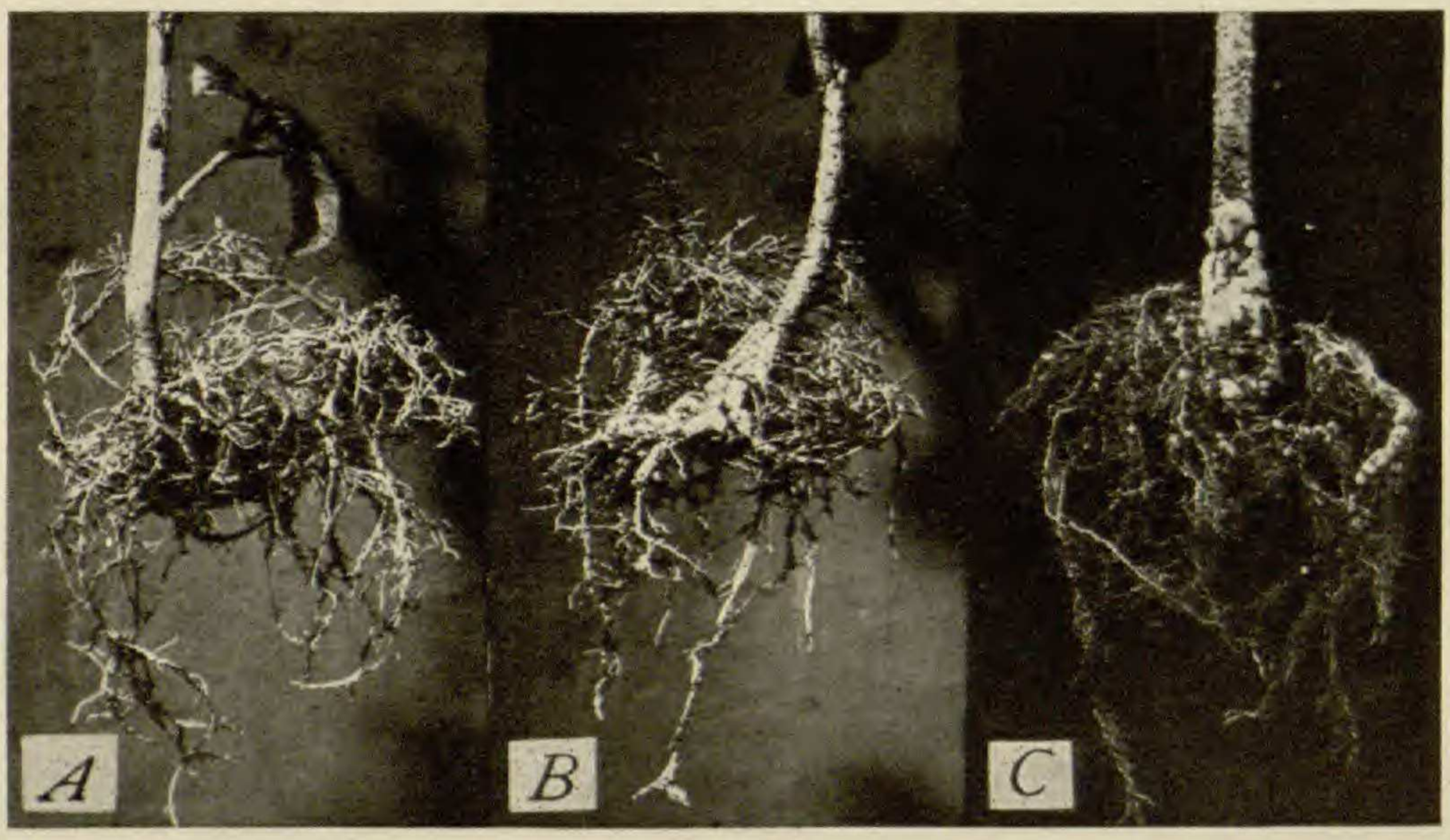


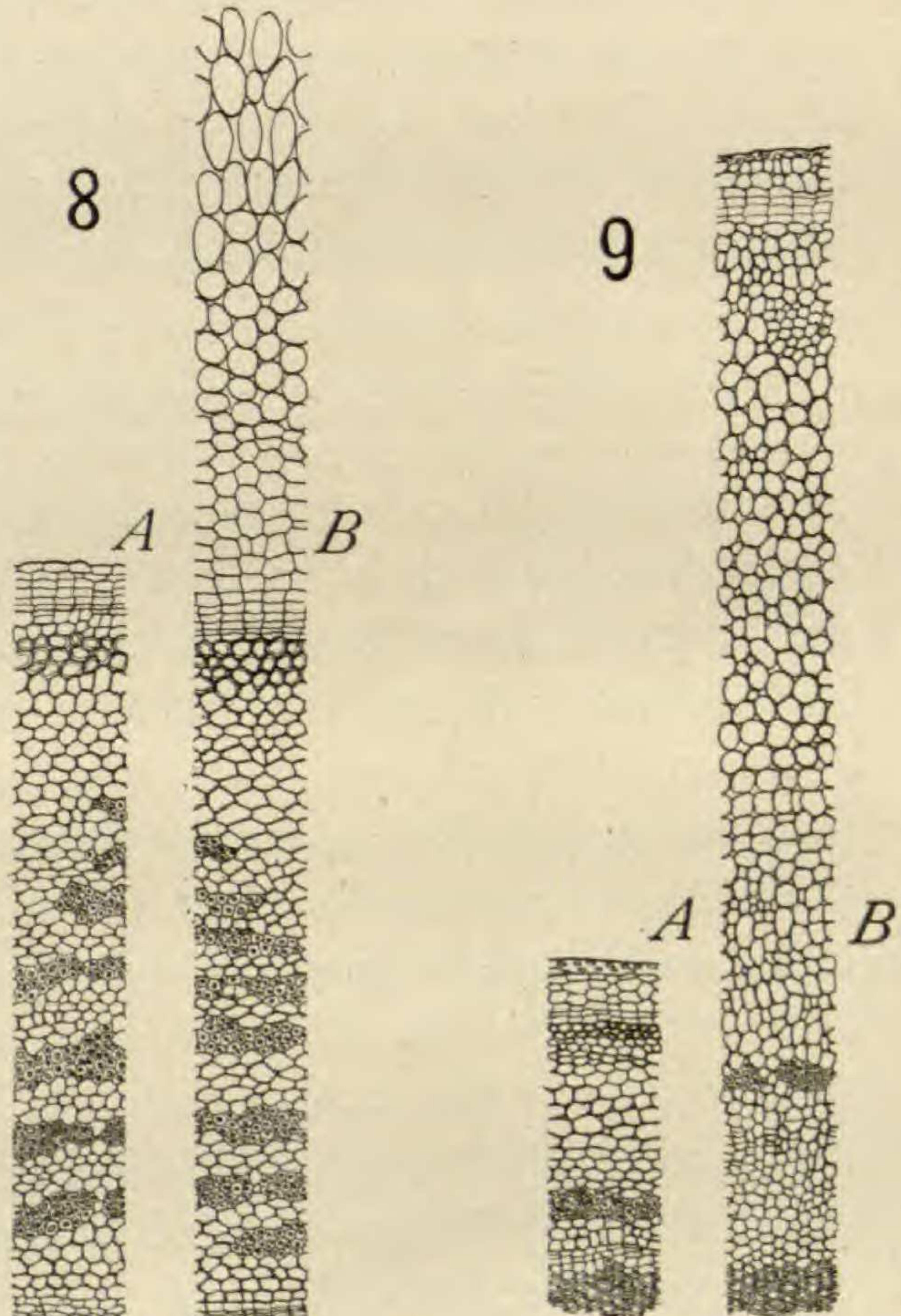
FIG. 7.—*Hibiscus: A*, control; *B*, *C*, treated with illuminating gas

layer of the abnormal root has been sloughed off. Practically the same morphological situation appears in this case as in *Ailanthus* previously figured.

A similar response by lilac is shown by drawings of fig. 9. These abnormalities were recorded for *Hibiscus*, lilac, *Croton*, *Diervilla*, *Ricinus*, *Ulmus*, and pear.

An experiment similar to the foregoing was carried out with an *Ailanthus* growing on the campus near the Hull Botanical Laboratory. The tree had a diameter of about 8 cm. and a height of 3.5 m. At a short distance were other trees of the same species which served as controls. Illuminating gas was admitted to the roots of the experimental tree through a glass tube thrust 0.7 m. into the soil, 0.6 m. from the base of the tree. The rate of flow of gas was 1.5 liters per hour. The experiment began July 3, and the gas was stopped flowing September 2. Except for two or three short periods, amounting in all to less than three days, the flow of gas was continuous during the two months. Thus the soil near the tree must have received 1.5–2 cubic meters of gas.

The first symptoms of injury were manifest July 14. Leaves of some of the young shoots, growing on the same side of the tree from which the gas entered the soil, showed signs of wilting. Three days later these leaves and others had shriveled and died, but remained attached to the shoots. In some cases only a portion of a leaf was injured. A few leaves of older branches also wilted, but there was no general effect evident throughout the tree at that time. In the middle of September the apparently unaffected leaves began to fall, and finally the tree was free from leaves much before those of the controls. In October the tree looked as though it were entirely dead, and when an examination was made, after it had been removed from the soil, such was found to be the case. A general dryness of the tissues was noted, but neither anatomical changes nor gas odors were detected in them.



FIGS. 8, 9.—Fig. 8, transverse sections through cortex of *Hibiscus* at base of stem; ×40; *A*, control; *B*, treated with illuminating gas; fig. 9, same for lilac; ×35.

During the gas flow, the odor of gas was evident only at the point where the tube entered the soil. In order to determine whether the gas might be detected by a more delicate means, the etiolated sweet pea seedling test, as described by KNIGHT and CROCKER (7) was tried. When the seedlings were 1.5–2 cm. high, the petri dishes containing the seedlings were taken to the *Ailanthus* tree, placed directly on the soil, and the 10-liter cans inverted over them. The cans, each with a dish of seedlings beneath, were numbered and placed, with reference to distance from the point of entrance of gas, as follows: no. 1, 5 cm., toward tree; no. 2, 0.6 m., at base of tree; no. 3, 0.8 m., also near tree but on side opposite no. 2; no. 4, 1.2 m., on side directly opposite point of entrance of gas; no. 5, 3 m., as no. 4. The cans were placed August 27 and observations were made three days later. The results were as follows: no. 1 gave no growth (observations refer to epicotyls only); no. 2, some growth, with a little swelling; no. 3, growth slightly reduced and diageotropic; no. 4, slender and straight, 9–10 cm. high (normal); no. 5, as no. 4. These results indicate that the pea seedling probably offers a rather delicate test for the presence of illuminating gas in the soil. In this case the injurious effect on the seedlings was very evident in those placed near the base of the tree, where, as stated before, no odor of gas could be detected in the customary manner.

In consideration of the great difference in behavior of a given plant when exposed to low and to high concentrations of illuminating gas, it seems appropriate to make the following suggestions with regard to the diagnosis of gas injuries. One reason why the foregoing abnormal tissue developments have not been recorded in trees killed by gas is probably the fact that examination was made for pathological symptoms in trees which have died or have become seriously injured. Such conditions would mean that the gas had been at their roots in too high concentration to allow the stimulating effects to enter. Sometimes the proliferation reported above was found in roots when no suggestion of injury could be observed in the aerial portions. Therefore, when one is attempting to diagnose with certainty a serious injury suspected to be due to gas, he ought also to make an examination of roots of other trees in the vicinity which have not yet shown injuries in the leaves, thereby perhaps adding one more line of evidence. Another change found to take place under the influence of low concentrations of illuminating gas was the disappearance of starch from the cortex of the roots, an observation in agreement with that of Richter (12) and others.

Summary

1. When illuminating gas is passed through soil, the odor-giving constituents of the gas are readily absorbed by the soil particles and strongly held.

2. These odorous substances are very slightly, if at all, toxic to roots of plants growing in a soil containing them.

3. The constituents of illuminating gas which remain in a gaseous state in the soil interstices are the chief cause of injury to root systems.

4. Among these constituents, ethylene is probably the most harmful, except in extremely high concentrations of illuminating gas, where the toxicity of other substances, together with other factors, would be expected to play a part.

5. Low concentrations of gas induce abnormal development of tissue.

a) Illuminating gas.—These abnormalities appear in certain tree seedlings within 8–21 days, with concentration one part illuminating gas to four parts air (air of the soil), or as low as one part illuminating gas to forty of air.

b) Ethylene.—This gas alone, when used in concentrations corresponding to the ethylene content of the illuminating gas used in the tests, gives abnormalities similar in type and degree.

6. High concentrations of illuminating gas result in the rapid killing of the roots, and the only symptom of injury to be observed is death.

7. If illuminating gas is allowed to flow very slowly through a soil in which woody plants are growing, abnormal tissue development in the root will very often ensue.

8. In low concentrations of illuminating gas, hydrolysis of starch and some other related chemical reactions are accelerated.

9. It was found that, by use of the etiolated sweet pea seedling, small amounts of illuminating gas in the soil could be detected where the odor of gas was indistinguishable by the usual methods.

We wish to acknowledge our indebtedness to Dr. WILLIAM CROCKER of this laboratory for many valuable suggestions during the course of this investigation.

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